Methoxymethyl-Directed Aryl Metalation. A Total Synthesis of (±)-Averufin[1]

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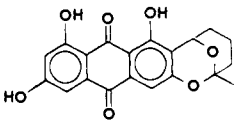
Abstract: A total synthesis of (±)-averufin, a central intermediate in aflatoxin biosynthesis, is described. The key steps of the synthesis involve the following: (1) the regiospecific coupling of the phthalide anion of **3b** and the benzyne derived in situ from aryl bromide **4** to give as a single isomer anthraquinone **5c**, the immediate precursor of the target molecule; and (2) the use of (methoxymethyl)phenol-protecting groups for regiospecific aryl metalation and introduction of electrophiles to selectively elaborate simple oxygenated benzenoid precursors.

Heavily oxygenated aromatic rings frequently constitute the major structural elements of polyketide-derived natural products.[2] The propensity of certain of these systems to rearrange, oxidatively couple, or decompose in acid or base makes their synthesis in fully unprotected, bioactive form a task of some difficulty. Methoxymethyl groups provide first a means to protect and ultimately deprotect hydroxyl functions under mild conditions. Second, as a means to elaborate simple, properly oxygenated benzenoid precursors to more complex systems, methoxymethyl is a highly serviceable directing group of intermediate strength[3] which allows regiospecific aryl metalation and subsequent reaction with a variety of electrophiles in good yield.[4] We report herein a total synthesis of averufin (**1**),[5,6] a pivotal intermediate in aflatoxin B_1 (**2**) biosynthesis,[7] which illustrates application of these concepts.



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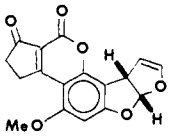

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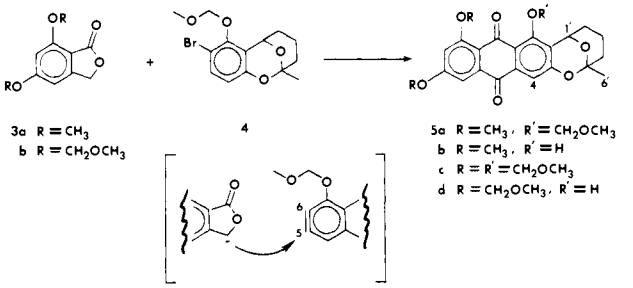
Extending the earlier work of Hauser,[8] Kraus[9] and Sammes,[10] we planned to couple in a convergent fashion two benzenoid precursors, the phthalide anion of **3** and the benzyne derived in situ from aryl bromide **4** to the triprotected anthraquinone **5**. It was anticipated that the latter could be deprotected under sufficiently mild conditions to averufin that the bicyclic ketal side chain would not be adversely affected.[5] Clearly success of the envisioned synthesis was dependent upon regiospecific addition of the anion of **3** to the benzyne at C-5 as shown. The basis for

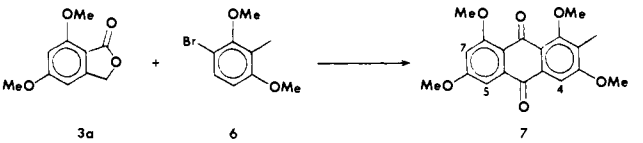


3a R = CH_3
b R = CH_2OCH_3

4

5a R = CH_3, R' = CH_2OCH_3
b R = CH_3, R' = H
c R = R' = CH_2OCH_3
d R = CH_2OCH_3, R' = H

this expectation was a priori the greater stability of the intermediate aryl anion formed at C-6 by reason of inductive and chelating effects[3] of the *o*-methoxymethyl group. The critical benzyne coupling reaction was therefore examined with the following model system. 5,7-Dimethoxyphthalide[11] (**3a**) was treated[10] (−60 °C, tetrahydrofuran) with 3 equiv of lithium 2,2,6,6-tetramethylpiperidide (LiTMP)[12] followed at −40 °C by 2 equiv of 3-bromo-2,6-dimethoxytoluene (**6**).[13] After aerial oxidation and silica gel chromatography, a 66% yield of the bright yellow anthraquinone **7**, mp 192 °C, was obtained as a *single* isomer.[14]



3a 6 7

(1) Presented at the 181st National Meeting of the American Chemical Society, Atlanta, GA, March–April, 1981; ORGN-17.

(2) Devon, T. K.; Scott, A. I. "Handbook of Naturally Occurring Compounds"; Academic Press: New York, 1975; Vol. 1.

(3) Gschwend, H. W.; Rodriguez, H. R. *Org. React. (N.Y.)* **1979**, *26*, 1–360.

(4) Stern, R.; English, J., Jr.; Cassidy, H. G. *J. Am. Chem. Soc.* **1957**, *79*, 5792–7. Christensen, H. *Synth. Commun.* **1975**, *5*, 65–78. Ronald, R. C. *Tetrahedron Lett.* **1975**, 3972–4; **1976**, 4413–6. Narasimhan, N. S.; Mali, R. S.; Barve, M. V. *Synthesis* **1979**, 906–9. Townsend, C. A.; Bloom, L. M. *Tetrahedron Lett.*, in press.

(5) Structure: Pusey, D. F. G.; Roberts, J. C. *J. Chem. Soc.* **1963**, 3542–7. Holker, J. S. E.; Kagal, S. A.; Mulheirn, L. J.; White, P. M. *J. Chem. Soc., Chem. Commun.* **1966**, 911–3. Roffey, P.; Sargent, M. V. *Ibid.* **1966**, 913–4. Roffey, P.; Sargent, M. V.; Knight, J. A. *J. Chem. Soc. C* **1967**, 2328–31.

(6) Synthesis: Castonguay, A.; Brassard, P. *Can. J. Chem.* **1977**, *55*, 1324–32. In this synthesis 1,3,6,8-tetrahydroxyanthraquinone was reacted with 5-oxohexanal in the presence of sodium bicaronate to give a 6.5% yield of averufin.

(7) Heathcote, J. G.; Hibbert, J. R. "Developments in Food Science. Aflatoxins: Chemical and Biological Aspects"; Elsevier: New York, 1978; Vol. 1, pp 151–72.

(8) Hauser, F. M.; Rhee, R. P. *J. Org. Chem.* **1978**, *43*, 178–80; *J. Am. Chem. Soc.* **1978**, *101*, 1628–9. Hauser, F. M.; Prasanna, S. *J. Org. Chem.* **1979**, *44*, 2596–8. See also: Russell, R. A.; Warrener, R. N. *J. Chem. Soc., Chem. Commun.* **1981**, 108–10.

(9) Kraus, G. A.; Sugimoto, H. *Tetrahedron Lett.* **1978**, 2263–6. Kraus, G. A.; Pezzanite, J. O. *J. Org. Chem.* **1979**, *44*, 2480–2.

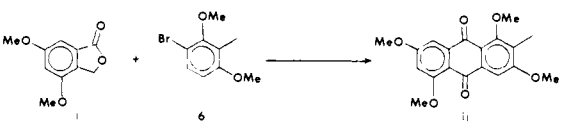
(10) Broom, N. J. P.; Sammes, P. G. *J. Chem. Soc., Chem. Commun.* **1978**, 162–4. Sammes, P. G.; Dodsworth, D. J. *Ibid.* **1979**, 33–4.

(11) Trost, B. M.; Ribers, G. T.; Gold, J. M. *J. Org. Chem.* **1980**, *45*, 1835–8.

(12) Olofson, R. A.; Dougherty, C. M. *J. Am. Chem. Soc.* **1973**, *95*, 582–4. Of several bases examined for this transformation LiTMP was clearly the best. For comparison, LDA[10] gave an 8% yield of anthraquinone.

(13) Schlegel, D. C.; Tipton, C. G.; Rinehart, K. L., Jr. *J. Org. Chem.* **1970**, *35*, 849. The corresponding iodo compound gave a lower yield of anthraquinone.

(14) The 1,3,6,8-tetramethoxy isomer **7** was prepared independently by two separate, unambiguous routes: S. B. Christensen (unpublished results); Matsuura, S.; Ohta, K. *Yakugaku Zasshi* **1962**, *82*, 959–6. All specimens of **7** were found to have mp 192 °C and identical spectral data. For comparison the benzyne coupling reaction described in the text was run with 4,6-dimethoxyphthalide i[15] and bromide **6** to give cleanly the isomeric 1,3,5,7-tetramethoxyanthraquinone ii, mp 219–220 °C, having chromatographic retentions and ^1H NMR chemical shifts distinct from those shown by **7**.



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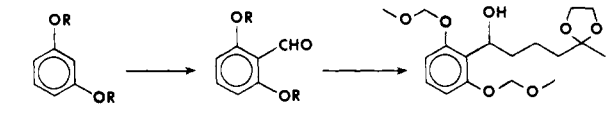
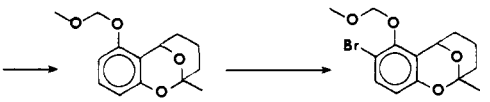
Heartened by this favorable outcome, we set about the synthesis of the key bromide **4**. Wittig had shown some years ago[16] that dimethylresorcinol (**8a**) could be metalated regiospecifically and reacted with *N*-methylformanilide to give aldehyde **9a** in 55% yield. Based on this precedent, bis(*O*-methoxymethyl)resorcinol (**8b**) was metalated and reacted with dimethylformamide at room temperature to give a 63% yield of aldehyde **9b**, mp 56.5–57.5 °C. The requisite C_5-Grignard reagent[17] was generated under Rieke[18] conditions and reacted with **9b** at room temperature in tetrahydrofuran overnight to afford an 87% yield of alcohol **10**. Cleavage of the methoxymethyl groups according to conventional practice in refluxing 50% aqueous acetic acid containing a trace of mineral acid resulted in instantaneous formation of polymer. Other attempts using strong acid and/or heat gave similar results. However, treatment as above but at room temperature for 2 days gave, after silica gel chromatography, 60–80% yields (range over several trials) of the pure, phenol-protected ketal **11**. Apparently, the benzylic hydroxyl assists the cleavage of one methoxymethyl group after which ketalization is spontaneous (intramolecular) and the second methoxymethyl group is essentially stable to the room-temperature hydrolysis conditions. The extreme acid lability of **11** made electrophilic bromination problematic. Our fears were



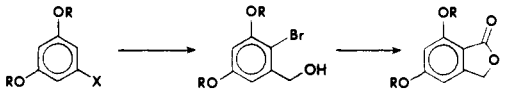
8a R = CH_3 **9a** R = CH_3 **10**
b R = CH_2OCH_3 **b** R = CH_2OCH_3



11 **4**

confirmed in each of several attempts. However, the potential problems of a mixture of isomeric bromides and the acid sensitivity of **11** were circumvented by regiospecific metalation of ketal **11** and reaction with cyanogen bromide[19] to give the desired bromide **4** free of isomeric contaminants in 95% yield after Kugelrohr distillation. Hence, two applications of methoxymethyl-directed aryl metalation generated the needed bromide efficiently and regiospecifically.

Reaction of dimethoxyphthalide **3a** with bromide **4** gave anthraquinone **5a** which appeared on thin-layer chromatography as a single yellow spot. On workup in dilute acetic acid this spot gradually decreased in intensity and was concomitantly replaced by a second, less-polar, anthraquinone product, **5b**, which had lost the 1-methoxymethyl.[20] Averufin (**1**) has been reported[5] to be stable to 50% hydrobromic acid in acetic acid at reflux for 4 h. Demethylation of **5b** under a variety of conditions, however, gave only low yields of averufin. It was apparent that more readily-removed protecting groups were needed for the A ring. To this end the corresponding bis(methoxymethoxy)phthalide (**3b**) was synthesized. Methyl 3,5-bis(methoxymethoxy)benzoate (**12**) was reduced to the benzylic alcohol **13** with lithium aluminum hydride. Metalation of **13** as carried out earlier in the synthesis of **3a**[11] occurred predominantly at C-4 with the desired phthalide **3b** being obtained in only 5% yield on quenching with dry carbon dioxide. Therefore, taking advantage of the normal course of electrophilic addition, **13** was treated with *N*-bromosuccinimide in chloroform[21] to afford bromide **14** (81%, mp 63.5–64 °C) which was transmetalated at −60 °C in tetrahydrofuran and reacted with carbon dioxide to afford the highly crystalline phthalide **3b** (30%, mp 96–97 °C).



12 X = COOMe **14** **3b**
13 X = CH_2OH R = CH_2OCH_3

Finally, reaction of **3b** with the previously obtained bromide **4** as above gave the triprotected anthraquinone **5c**. In accord with expectation this compound readily hydrolyzed on aqueous workup to a mixture of the tri- and diprotected[22] anthraquinones **5c** and **5d** isolated in about 35% yield by silica gel chromatography. As hoped, deprotection proceeded smoothly either in hot 50% aqueous acetic acid containing a trace of concentrated sulfuric acid or in methanol containing 5% concentrated hydrochloric acid to give (±)-averufin (**1**), 80% yield (8% overall from starting ester **12**), having retentions and fluorescence behavior on thin-layer chromatography, physical, and spectral data identical with those of authentic material.

Experimental Section

Melting points were determined in open capillaries with a Thomas-Hoover apparatus and are uncorrected. Spectral data were collected with use of the following spectrometers and solvents: UV–vis, Cary Model 219 for solutions in absolute ethanol (positions of absorptions given in nm); IR, Perkin-Elmer Model 599B for solutions in chloroform unless otherwise noted (band positions reported in reciprocal centimeters, cm^{-1}); ^1H NMR, Varian CFT-20 fitted with a proton probe to operate at 80 MHz, or as noted a Bruker WM 300, for solutions in deuteriochloroform, except as otherwise indicated (chemical shifts recorded in ppm relative to tetramethylsilane as internal standard, homonuclear coupling constants in Hz). Microanalyses were performed by Galbraith Laboratories, Knoxville, TN. Thin-layer chromatography (TLC) was carried out with Analtech glass plates coated (0.25 mm) with silica gel (GHLF Uniplate) and preparative layer chromatography (PLC) with 20 × 20 cm plates, 0.5 and 2.0 mm thickness, of E. Merck silica gel F-254. Open-column chromatography was conducted with silica gel-60, 0.063–0.200 mm, E. Merck. Distillations of liquid products were performed with a Büchi Kugelrohr oven at the indicated air bath temperature and pressure. Tetrahydrofuran (THF) and ether were distilled from sodium benzophenone ketyl immediately before use. Dimethylformamide (DMF) was distilled under reduced pressure from calcium hydride. 2,2,6,6-Tetramethylpiperidine (TMP) and diisopropylamine were distilled from and stored over potassium hydroxide pellets. Prior to commencing reactions, apparatuses were flamed dry while purging with dry nitrogen. All reactions were carried out under dry nitrogen.

1,3-Bis(*O*-methoxymethyl)resorcinol (8b). A 500 mL, three-neck, round-bottom flask was fitted with a reflux condenser, addition funnel, and thermometer and charged with sodium hydride (50% dispersion in mineral oil, 25.0 g, 0.52 mmol). The mineral oil was removed with hexane (3 × 10 mL), DMF (130 mL) was added, and a solution of resorcinol (25.0 g, 0.23 mmol) was added slowly in DMF (100 mL) to maintain a reaction temperature of less than 75 °C. After being stirred for 2 h at room temperature, chloromethyl methyl ether (Aldrich, distilled, 38.4 mL, 41 g, 0.51 mmol) was added dropwise at such a rate as

(15) Brockmann, H.; Kluge, F.; Muxfeldt, H. *Chem. Ber.* **1957**, *90*, 2302–18.
(16) Wittig, G. "Newer Methods of Preparative Organic Chemistry"; Interscience: New York, 1948; p 279.
(17) Crombie, L.; Hemesley, P.; Pattenden, G. *J. Chem. Soc C* **1969**, 1016–24. Weyerstahl, P.; Zummack, W. *Chem. Ber.* **1975**, *108*, 377–8.
(18) Rieke, R. D.; Hudnall, P. M. *J. Am. Chem. Soc.* **1972**, *94*, 7178–9. Rieke, R. D.; Bales, S. E. *Ibid.* **1974**, 1775–81.
(19) Boltze, K.-H.; Dell, H.-D.; Janzen, H. *Ann.* **1967**, *709*, 63–9. Essentially complete conversion to the bromide could only be accomplished in rigorously dried ether; in the present case it was obtained by distillation from *freshly* prepared sodium benzophenone ketyl. For an alternative source of bromine see: Ronald, R. C.; Gewali, M. B.; Ronald, B. P. *J. Org. Chem.* **1980**, *45*, 2224–9.
(20) The enhanced acid lability of protecting groups at the C-1 hydroxyl in 2-substituted anthraquinones had been observed throughout our work. Their loss was readily confirmed by an upfield shift of approximately 0.5 ppm for the singlet corresponding to H-4 in the ^1H NMR accompanied by the appearance of the intramolecularly hydrogen-bonded 1-OH at >12 ppm which in both **5b** and **5d** appeared at 13.55 ppm.
(21) Bromination in carbon tetrachloride gave products of electrophilic addition at C-2 and C-4 in a ratio of roughly 4:1, respectively.
(22) Some 1,6-bis(methoxymethoxy)averufin was obtained as well (see Experimental Section).
(23) Aucamp, P. J.; Holzapfel, C. W. *J. S. Afr. Chem. Inst.* **1970**, *23*, 40–56.
(24) Donkersloot, J. A.; Mateles, R. I.; Yang, S. S. *Biochem. Biophys. Res. Commun.* **1972**, *47*, 1051–5.

to keep the reaction temperature below 60 °C. When the addition was complete, the mixture was stirred 3 h and then stirred open to the air (hood) overnight. The reaction was quenched with cold water (750 mL), filtered, and extracted with benzene (4 × 300 mL). The organic extracts were pooled, washed with 5% sodium hydroxide (3 × 300 mL), water (2 × 300 mL), and brine (2 × 200 mL), and dried over anhydrous potassium carbonate. The solvent was removed in vacuo and the resulting oil was distilled under reduced pressure to give **8b** as a colorless, viscous oil (36.11 g, 81%, bp 137 °C (7 torr)). IR 3030, 2960, 2830, 2780, 1605, 1595, 1485, 1400, 1260, 1150, 1080, 1020 cm^{-1}; ^1H NMR δ 3.47 (s, 6 H), 5.15 (s, 4 H), 6.6–6.75 (cm, 3 H, sharp line at 6.74), 7.1–7.3 (cm, 1 H). Anal. ($C_{10}H_{14}O_4$): C, H.

2,6-Bis(methoxymethoxy)benzaldehyde (9b). To a 250-mL, three-neck, round-bottom flask equipped with a reflux condenser, addition funnel, and nitrogen inlet was added 47 mL of dry ether followed by *n*-butyllithium in hexane (20.0 mL, 3.0 mmol). A solution of 1,3-bis(*O*-methoxymethyl)resorcinol (**8b**, 5.26 g, 26.6 mmol) in dry ether (5 mL) was added dropwise over 15 min and the mixture was heated to reflux for 1 h. After being cooled to room temperature, dry DMF (2.7 mL, 2.54 g, 35.0 mmol) was added dropwise to the milky reaction mixture (exothermic). The mixture was stirred 0.5 h and quenched by the addition of cold water. The reaction mixture was partitioned between ether and water and the aqueous layer was extracted three times with ether. The combined organic extracts were washed with saturated ammonium chloride (3 × 40 mL), 5% sodium hydroxide (2 × 40 mL), water (3 × 40 mL), and brine and dried over anhydrous potassium carbonate. Evaporation of the solvent under reduced pressure gave a yellow oil (4.49 g) which solidified on standing. Recrystallization (ether/hexanes) afforded 2,6-bis(methoxymethoxy)benzaldehyde (**9b**) as very pale yellow needles (3.78 g, 63%, mp 56.5–57.5 °C). IR 3020, 2960, 2830, 2780, 1685, 1595, 1470, 1400, 1260, 1165, 1050 cm^{-1}; ^1H NMR δ 3.50 (s, 6 H), 5.26 (s, 4 H), 6.81 (d, J_{app} = 8 Hz, 2 H, AB_2), 7.40 (split t, J_{app} = 8 Hz, 1 H AB_2), 10.54 (s, 1 H). Anal. ($C_{11}H_{14}O_5$): C, H.

Tricyclic Ketal 11. Activated magnesium was prepared according to Rieke's procedure[18] from anhydrous magnesium chloride (Aldrich) that had been further dried for 120 h under vacuum in a drying pistol (xylene) over phosphorus pentoxide. So prepared anhydrous magnesium chloride (5.6 g, 58.5 mmol) in dry THF (125 mL) was heated to reflux under nitrogen for 3 h in the presence of potassium metal (4.30 g, 107.7 mmol). After being cooled to room temperature, 5-bromo-2-pentanone ethylene ketal (4.3 mL, 5.76 g, 27.6 mmol)[17] was added and the black mixture was stirred 3 h at room temperature whereupon 2,6-bis(methoxymethoxy)benzaldehyde (**9b**, 5.6 g, 24.8 mmol) was added. The reaction mixture was stirred overnight [TLC (ether) showed disappearance of starting aldehyde **9b**, R_f 0.66, and the appearance of alcohol as essentially the sole product, R_f 0.46] and quenched by the addition of cold water. The product was isolated by successive extractions with ether (4 × 150 mL). The combined extracts were washed with water (2 × 150 mL) and brine and dried over anhydrous potassium carbonate. Removal of the solvent in vacuo gave crude benzylic alcohol **10** as a pale yellow oil [7.6 g, 87%; ^1H NMR δ 1.3–2.0 (cm, 6 H), 1.30 (s, 3 H), 3.48 (s, 6 H), 3.90 (s, 4 H), 5.15 (obscured br t, 1 H), 5.20 (s, 4 H), 6.72–6.83 (split d, J_{app} ~ 8 Hz, 2 H, AB_2), 7.02–7.23 (4 lines, 1 H, AB_2)]. Alcohol **10** was found to be unstable on standing and so was converted directly to tricyclic ketal **11** in 50% aqueous acetic acid (v/v) containing one drop of concentrated sulfuric acid (100 mL, degassed 1 h with nitrogen ebullition) at room temperature for 50 h under a positive nitrogen pressure. Water was added (300 mL) and the cloudy mixture was extracted with ether (4 × 150 mL) and the combined organic extracts were washed with water (2 × 200 mL), 5% sodium bicarbonate (1 × 200 mL), and brine (2 × 150 mL) and dried over anhydrous potassium carbonate. Concentration in vacuo gave a dark yellow oil from which pure ketal **11** was isolated by chromatography on 150 g of silica gel (hexane:ether 4:1) and Kugelrohr distillation (140 °C (1 torr)) as a colorless, viscous oil (4.2 g, 79% from crude alcohol **10**). It was later found that this oil crystallized on prolonged standing in the cold, mp 45.5–47 °C. IR 3000, 2950, 2870, 2850, 2820, 2790, 1610, 1595, 1470, 1245, 1160, 1050 cm^{-1}; ^1H NMR δ 1.2–2.2 (cm, 6 H), 1.52 (s, 3 H), 3.44 (s, 3 H), 5.16 (s, 2 H), 5.26 (br d, J_{app} ~ 3 Hz, 1 H), 6.48 (d, J = 8 Hz, 1 H, broadened by ~1 Hz by 1,3-coupling), 6.57 (d, J = 8 Hz, 1 H, broadened by ~1 Hz), 7.07 (t, J = 8, 1 H). Anal. ($C_{14}H_{18}O_4$): C, H.

Tricyclic Ketal Bromide 4. *n*-Butyllithium (1.0 mL, 1.38 mmol) in hexane was added to a solution of tricyclic ketal methoxymethyl ether **11** (225 mg, 0.896 mmol) in 13 mL of dry ether.[19] The initially bright yellow solution after 0.7–1.0 h developed a milky white precipitate. After being stirred a total of 3.5 h at room temperature, a 2.4 M solution of cyanogen bromide in dry ether (1.0 mL, 2.4 mmol) was added whereupon the mixture immediately became light brown. After 0.5 h, water (20 mL) was added and the layers were separated. The aqueous phase was extracted with ether (2 × 10 mL) and the organic extracts were combined, washed with brine (2 × 15 mL), and dried over anhydrous potassium carbonate. Removal of the ether under reduced pressure yielded a light brown oil which was purified by Kugelrohr distillation (120 °C (1 torr)) to afford pure, colorless bromide **4** as a viscous oil (282 mg, 95%). IR 3000, 2940, 1595, 1580, 1470, 1170, 1030, 950, 680 cm^{-1}; ^1H NMR δ 1.2–2.2 (cm, 6 H), 1.52 (s, 3 H), 3.57 (s, 3 H), 5.13 (s, 2 H), 5.37 (br t, 1 H), 6.53 (d, J = 8 Hz, 1 H), 7.31 (d, J = 8 Hz, 1 H). Anal. ($C_{14}H_{17}O_4Br$): C, H.

Methyl 3,5-Bis(methoxymethoxy)benzoate (12). Methyl 3,5-dihydroxybenzoate (5.0 g, 29.8 mmol) in 30 mL of DMF was added to sodium hydride (from 3.3 g of 50% dispersion in mineral oil, 69 mmol) suspended in 70 mL of DMF. Addition was carried out to maintain the temperature of the mixture at <40 °C. After 2 h, chloromethyl methyl ether (5.7 mL, 5.8 g, 71.0 mmol) was added at a rate to keep the temperature of the reaction at <50 °C. The remainder of the reaction and workup was conducted as earlier for **8b**. Kugelrohr distillation (155 °C (1 torr)) gave pure methyl 3,5-bis(methoxymethoxy)benzoate (**12**) as a colorless liquid (6.5 g, 85%). IR 3030, 3000, 2950, 2900, 2820, 1730, 1600, 1580, 1435, 1300, 1220, 1150, 1035 cm^{-1}; ^1H NMR δ 3.48 (s, 6 H), 3.89 (s, 3 H), 5.18 (s, 4 H), 6.91 (t, J = 2.3 Hz, 1 H), 7.36 (d, J = 2.3 Hz, 2 H). Anal. ($C_{12}H_{16}O_6$): C, H.

3,5-Bis(methoxymethoxy)benzyl Alcohol (13). A solution of methyl 3,5-bis(methoxymethoxy)benzoate (**12**, 1.09 g, 4.26 mmol) in 10 mL of THF was added in a dropwise fashion to a suspension of lithium aluminum hydride (0.52 g, 13.7 mmol) in 35 mL of dry THF. After 2 h, the reaction was quenched by the careful addition of 5 mL of water and 5 mL of 15% (w/v) aqueous sodium hydroxide. The precipitated salts were removed by filtration and 100 mL of water were added to the filtrate which was extracted with ether (3 × 50 mL). The combined organic extracts were washed with brine, dried over anhydrous potassium carbonate, and concentrated in vacuo. Kugelrohr distillation (135 °C (1 torr)) of the resulting oil gave benzylic alcohol **13** as a colorless liquid (0.95 g, 98%). IR 3600, 3460, 3000, 2950, 2900, 2820, 2780, 1600, 1460, 1290, 1150, 1085, 1030, 925 cm^{-1}; ^1H NMR δ 2.0 (br s, 1 H, –O*H*), 3.46 (s, 6 H), 4.61 (s, 2 H), 5.15 (s, 4 H), 6.69 (br split s, 3 H). Anal. ($C_{11}H_{16}O_5$): C, H.

2-Bromo-3,5-Bis(methoxymethoxy)benzyl Alcohol (14). *N*-Bromosuccinimide (234 mg, 1.31 mmol) was added as a solid to a solution of 3,5-bis(methoxymethoxy)benzyl alcohol (**13**, 298 mg, 1.31 mmol) in 30 mL of chloroform at 45 °C. The solution became yellow and was stirred at 45 °C for 0.5 h. The chloroform solution was washed with water (10 × 10 mL), dried over anhydrous potassium carbonate, and concentrated in vacuo to afford bromide **14** as a white crystalline solid (337 mg, 83%). Recrystallization from chloroform/hexanes gave needles (81%, mp 63.5–64.0 °C). IR 3600, 3460, 3000, 2960, 2900, 2830, 2780, 1590, 1450, 1320, 1150, 1140, 1020, 930 cm^{-1}; ^1H NMR δ 2.1 (br s, 1 H, –O*H*), 3.47 (s, 3 H), 3.51 (s, 3 H), 4.73 (br s, 2 H), 5.16 (s, 2 H), 5.23 (s, 2 H), 6.80 (d, J = 2.7 Hz, 1 H), 6.91 (d, J = 2.7 Hz, 1 H). Anal. ($C_{11}H_{15}O_5Br$): C, H, Br.

5,7-Bis(methoxymethoxy)phthalide (3b). 2-Bromo-3,5-bis(methoxymethoxy)benzyl alcohol (**13**, 94 mg, 0.30 mmol) in 10 mL of THF at –60 °C (methylene chloride/dry ice bath) was treated with *sec*-butyllithium (970 μL, 1.22 mmol) in cyclohexane. The solution became pale yellow and was stirred for 0.5 h after which time excess carbon dioxide was bubbled through the solution for 0.5 h. The bath was removed and the mixture was stirred an additional 0.5 h and then quenched by the addition of 25 mL of water. The aqueous solution was extracted with ether (3 × 15 mL). The combined organic extracts were washed with brine (3 × 10 mL), dried over anhydrous potassium carbonate, and concentrated in vacuo to afford 3,5-bis(methoxymethoxy)benzyl alcohol (**13**, 37.1 mg, 56%). The aqueous layer was acidified to pH 4 with 1 N hydrochloric acid and extracted with ethyl acetate (3 × 15 mL). The combined organic layers were washed with brine (3 × 10 mL), dried over anhydrous potassium carbonate, and evaporated to give a light brown oil (39.0 mg) which was purified by TLC (ether) to afford phthalide **3b** (22.6 mg, 30%, 68% based on recovered alcohol **13**) which was recrystallized from acetone/hexanes, mp 96–97 °C. IR 3030, 2960, 2940, 2830, 2790, 1750, 1610, 1480, 1330, 1150, 1080, 1000 cm^{-1}; ^1H NMR δ 3.49 (s, 3 H), 3.59 (s, 3 H), 5.11 (s, 2 H, phthalide methylene), 5.23 (s, 2 H), 5.34 (s, 2 H), 6.74 (d, J ~ 1 Hz, 1 H), 6.82 (d, J ~ 1 Hz, 1 H). Anal. ($C_{12}H_{14}O_6$): C, H.

2-Methyl-1,3,6,8-tetramethoxyanthraquinone (7). 2,2,6,6-Tetramethylpiperidine (135 μL, 113 mg, 0.80 mmol)[12] in 0.5 mL of THF was treated at –60 °C (methylene chloride/dry ice bath) with *n*-butyllithium in hexane (600 μL, 0.84 mmol). After being stirred for 10 min, 5,7-dimethoxyphthalide[11] (**3a**, 50.0 mg, 0.258 mmol) in 4.5 mL of dry THF was added. After 20 min the orange solution was warmed to –40 °C[10] (acetonitrile/dry ice bath) and a solution of 3-bromo-2,6-dimethoxytoluene[13] (**6**, 125.9 mg, 0.545 mmol) in 2 mL of THF was added. After being stirred 15 min, the mixture was allowed to come gradually to room

temperature, the color becoming dark red to purple, and after 1 h, the mixture was stirred open to the air for at least 4 h. Addition of water (30 mL) and extraction of the resulting mixture with chloroform (3 × 8 mL), washing of the combined organic extracts with brine, drying over anhydrous magnesium sulfate, and removal of the solvents in vacuo gave the crude anthraquinone. Purification of the yellow solid by column chromatography on silica gel (3.0 g, ethyl acetate:hexanes 1:1) gave 2-methyl-1,3,6,8-tetramethoxyanthraquinone (**7**, 60.0 mg, 66%). Recrystallization from ethyl acetate/hexanes gave fine yellow needles (56 mg, 62%, mp 192 °C (lit.[14] mp 192 °C)). IR 3050, 2980, 2940, 2870, 1665, 1600, 1580, 1460, 1325, 1265, 1165, 1140, 1000 cm^{-1}; ^1H NMR δ 2.24 (s, 3 H), 3.92 (s, 3 H), 3.95 (s, 3 H), 3.96 (s, 3 H), 3.98 (s, 3 H), 6.76 (d, J = 2.5 Hz, 1 H, H-7), 7.33 (d, J = 2.5 Hz, 1 H, H-5), 7.48 (s, 1 H, H-4).

As noted in ref 14, in order to establish unequivocally the regioselectivity of the benzyne coupling reaction, isomeric 4,6-dimethoxyphthalide (i)[15] (63.7 mg, 0.31 mmol) was reacted as above except with use of LDA[12] as base with 3-bromo-2,6-dimethoxytoluene (**6**)[13] to give the isomeric 2-methyl-1,3,5,7-tetramethoxyanthraquinone (ii) after silica gel chromatography (as above) and crystallization from ethanol/water (34 mg, 32%, mp 219–220 °C). IR, band positions same as for **7** but differ in relative intensities and shape; ^1H NMR δ 2.23 (s, 3 H), 3.89 (s, 3 H), 3.97 (s, 3 H), 3.99 (s, 6 H), 6.71 (d, J = 2.5 Hz, 1 H, H-6), 7.43 (d, J = 2.5 Hz, 1 H, H-8), 7.60 (s, 1 H, H-4).

(±)-6,8-Dimethoxyaverufin (5b). 5,7-Dimethoxyphthalide (**3a**, 101.5 mg, 0.51 mmol) in 10 mL of THF was reacted with 3.15 equiv of LiTMP in 1 mL of THF as for **7** above for 20 min at −60 °C. The temperature was raised to −40 °C and tricyclic ketal bromide **4** (337 mg, 1.02 mmol) and a total of 4 mL of THF was added in a dropwise manner. The solution turned bright red to purple over 40 min at which time the bath was removed and the reaction mixture was allowed to come to room temperature. The flask was opened to the air and glacial acetic acid was added (8 drops) and stirring was continued overnight. Water (50 mL) was added and the reaction mixture was extracted with chloroform (2 × 20 mL). The pooled organic extracts were washed with 5% aqueous sodium bicarbonate (2 × 15 mL), water (2 × 15 mL), and brine. After being dried over anhydrous magnesium sulfate, the solvents were removed under reduced pressure and the crude product was chromatographed on silica gel (5 g, ethyl acetate:hexanes 1:1) to give two yellow anthraquinone-containing fractions. The first gave cleanly the less polar (±)-6,8-dimethoxyaverufin (**5b**)[20] as bright orange fine crystals on recrystallization from chloroform/hexanes (40.7 mg, 19.6%, mp 211.5–212.5 °C). UV λ (log ε) 224 (4.52), 250 (4.08), 287 (4.43), 310 (3.88), 440 (3.89) nm; IR 3000, 2950, 2840, 1670 (weak), 1620, 1595, 1560, 1400, 1345, 1325, 1270, 1250, 1215, 1160, 995, 840 cm^{-1}; ^1H NMR δ 1.2–2.2 (cm, 6 H), 1.57 (s, 3 H), 3.97 (s, 3 H), 4.01 (s, 3 H), 5.39 (br t, 1 H), 6.77 (d, J = 2.3 Hz, 1 H, H-7), 7.20 (s, 1 H, H-4), 7.44 (d, J = 2.3 Hz, 1 H, H-5), 13.55 (s, 1 H, 1-O*H*). Anal. ($C_{22}H_{20}O_7$): C, H.

The second fraction contained a mixture of tri- and diprotected anthraquinones **5a** and **5b**, respectively (85.1 mg, ca. 35%). Owing to hydrolysis on silica gel,[20] it was not possible to isolate **5a** in a pure state. The mixture could be hydrolyzed in 50% aqueous acetic acid (v/v) containing a trace of concentrated sulfuric acid to give essentially pure **5b**.

(±)-Averufin (1). 5,7-Bis(methoxymethoxy)phthalide (**3b**, 114.3 mg, 0.45 mmol) was reacted with tricyclic ketal bromide **4** (282.2 mg, 0.86 mmol) under conditions employed above for the synthesis of 6,8-dimethoxyaverufin (**5b**). After aerial oxidation overnight, 50 mL of water were added and the mixture was extracted with ethyl acetate (3 × 30 mL). The combined organic extracts were washed with water (2 × 30 mL) and brine (2 × 30 mL) and dried over anhydrous potassium carbonate. Removal of the solvents at reduced pressure gave a yellow oil. TLC analysis (silica, hexane:ethyl acetate 3:2) indicated three anthraquinone products (none of which could be isolated in pure form[20]). Triprotected averufin **5c** as the principal component (R_f 0.43): ^1H NMR (300 MHz) δ 1.2–2.2 (cm, 6 H), 1.57 (s, 3 H), 3.50 (s, 3 H), 3.56 (s, 6 H), 5.29 (s, 4 H), 5.33 (s, 2 H), 7.13 (d, J = 2.5 Hz, 1 H, H-7), 7.47 (s, 1 H, H-4), 7.57 (d, J = 2.5 Hz, 1 H, H-5). Diprotected averufin **5d** (R_f 0.66): ^1H NMR (300 MHz) δ 1.2–2.2 (cm, 6 H), 1.57 (s, 3 H), 3.51 (s, 3 H), 3.58 (s, 3 H), 5.31 (s, 3 H), 5.38 (s, 3 H), 7.16 (d, J = 2.5 Hz, 1 H, H-7), 7.22 (s, 1 H, H-4), 7.64 (d, J = 2.5 Hz, 1 H, H-5), 13.55 (s, 1 H, 1-O*H*). A trace of a third compound (R_f 0.72) tentatively identified as the isomeric diprotected species 1,6-bis(methoxymethoxy)averufin: ^1H NMR (300 MHz) δ 1.2–2.2 (cm, 6 H), 1.59 (s, 3 H), 3.49 (s, 3 H), 3.59 (s, 3 H), 5.28 (s, 4 H), 6.87 (d, J = 2.5 Hz, 1 H, H-7), 7.40 (d, J = 2.5 Hz, 1 H, H-5), 7.55 (s, 1 H, H-4), 13.21 (s, 1 H, 8-O*H*). Column chromatography of the crude reaction mixture on silica gel (15 g, hexane:ethyl acetate 1:1) gave the anthraquinone product as a mixture of protected forms (72 mg, ca. 35%). In order to examine the complete removal of methoxymethyl protecting groups, one fraction from the column chromatography above which contained largely 6,8-bis(methoxymethoxy)averufin (**5d**, 23.2 mg, 0.051 mmol), after isolation as an orange solid, was treated with 50% aqueous acetic acid (v/v) containing a trace of sulfuric acid (1 drop/100 mL) (5 mL, purged with nitrogen) at 85 °C under nitrogen. After 10 h, 15 mL of water were added, the aqueous mixture was extracted with ethyl acetate (2 × 20 mL), and the dark orange organic extracts were washed with 5% sodium bicarbonate (2 × 15 mL), water (2 × 15 mL), and brine and dried over anhydrous magnesium sulfate. Removal of the solvents afforded 19.7 mg of brown solid. The highly polar dark components were removed by chromatography on a short column of silica gel (1 g, chloroform:methanol 97:3) to give (±)-averufin (**1**) as a yellow-orange crystalline solid (15.0 mg, 80%, mp >279 °C dec, (lit.[6] (synthetic) mp >280 °C dec, lit.[5] (natural) mp 280–282 °C dec, 280 °C dec,[23] 283–289 °C dec[24]). Material recrystallized from chloroform/hexanes was with respect to its behavior on TLC and physical and spectral characteristics identical with authentic material. In like fashion, the variously protected averufins isolated from the remaining chromatographic fractions could be readily hydrolyzed to (±)-averufin. UV λ_{max} (log ε) 224 (4.48), 254 (sh, 4.17), 264 (4.23), 294 (4.39), 316 (4.36), 450 (sh, 3.92), 467 (3.94) nm; IR (KBr) 3400 (br), 2975, 1670 (weak), 1620, 1570, 1400, 1315, 1270, 1210, 1160, 1025, 835 cm^{-1}; ^1H NMR ("100%" Me_2SO-d_6) δ 1.2–2.2 (cm, 6 H), 1.53 (s, 3 H), 5.24 (br s, 1 H, 1′-methine), 6.53 (d, J = 2.3 Hz, 1 H, H-7), 6.94 (s, 1 H, H-4), 7.05 (d, J = 2.3 Hz, 1 H, H-5), 8.3 (s, 1 H, 6-O*H*), 12.0 (br s, 1 H, 8- or 1-O*H*), 12.4 (br s, 1 H, 1- or 8-O*H*).

Acknowledgment. We thank J. C. Vederas (University of Alberta), D. P. H. Hsieh (University of California, Davis), P. Brassard (Université Laval), and P. S. Steyn (CSIR, Pretoria) for kindly providing samples of averufin. The financial support of the National Institutes of Health (5 R01 ES01670 and 5 S07 RR07041) and the donors of the Petroleum Research Fund, Administered by the American Chemical Society, is gratefully acknowledged. The Bruker WM 300 used in this work was purchased with funds in part provided by the National Institutes of Health (1P41 GM27512).